

**P116****Novel ECM-based Scaffolds for Cartilage Repair**C. Nataraj<sup>1</sup>, A. Alexander<sup>2</sup>, G. Ritter<sup>2</sup>, K.R. Zaslav<sup>3</sup>;<sup>1</sup>Research & Development, Pegasus Biologics Inc, Alachua, Florida, United States of America, <sup>2</sup>Research & Development, Pegasus Biologics Inc, Alachua, FL, United States of America, <sup>3</sup>Sports Medicine Center, Advanced Orthopedic Centers, Richmond, Virginia, United States of America**Purpose:** Tissue engineering technologies have increased treatment options for cartilage repair. Central to their success is the availability of an ideal scaffold. We describe here a novel technology to prepare a safe and effective biological scaffold.**Methods and Materials:** Using proprietary carbodiimide (EDC)-based technologies collagenous scaffolds (with native and/or reconstituted Type I collagen) were stabilized and sterilized. Additionally, using these technologies we stably attached ECM adjuncts (GAGs, Growth Factors) to the matrices. Stability, sterility and compatibility of these scaffolds were tested in vitro.**Results:** DSC and resistance to enzyme digestion (pronase, collagenase) confirmed the stability of the matrices, which remained unaffected by the subsequent sterilization. Effectiveness of sterilization was demonstrated by inactivation of indicator bacterial, viral and spore forming pathogens. Attachment of ECM adjuncts were validated using ELISA and differential histology (PAS-Alcian Blue). These tests also indicated stability of the adjuncts after sterilization and over time (up to 4 weeks in culture). Compatibility of the matrices was assessed by viability of primary chondrocytes from human, equine and bovine sources incubated on the matrix in static cultures. Cells maintained both viability and phenotypes over 4 weeks, and addition of adjuncts improved cell attachment and retention.**Conclusions:** The methodology presented here to generate scaffolds for cartilage repair allowed for both stability of the matrix and controlled release of chondrogenic adjuncts without being negatively affected by the sterilization method. Prolonged chondrocyte viability, maintenance of phenotype, and histological evidence of new matrix synthesis following cell culture, indicates the potential value of these scaffolds in cartilage repair.**P117****Composite non-woven scaffolds containing PGA, PVA, and chitosan improve pH stability and cell proliferation**E. Filova<sup>1</sup>, M. Rampichová<sup>1</sup>, E. Košťáková<sup>2</sup>, D. Lukáš<sup>2</sup>, A. Lytvynets<sup>3</sup>, E. Amler<sup>1</sup>;<sup>1</sup>Department Of Tissue Engineering, Institute of Experimental Medicine of the Academy of Sciences of the Czech Republic, Prague, Czech Republic, <sup>2</sup>Department Of Nonwoven Textiles, Faculty of Textile Engineering, Technical University of Liberec, Liberec, Czech Republic, <sup>3</sup>Department Of Animal Breeding And Hygiene, Institute of Physiology of the Academy of Sciences of the Czech Republic, Prague, Czech Republic**Purpose:** Scaffolds from poly (glycolic) acid promote cell proliferation, however the decrease of pH during their degradation may cause inflammation. The aim of this study was to develop composite non-woven scaffolds possessing better pH stability.**Methods and Materials:** Different non-woven scaffolds based on poly(glycolic) acid (PGA) and poly(vinyl) alcohol (PGA/PVA and PVA/PVA), hyaluronic acid (PGA/HA I and PGA/HA II), and chitosan (PGA/PVA/CH) were prepared. The samples of six-mm diameter were incubated in 20 mL of PBS at 37 °C for 42 days, and pH was measured every other day. Weight (both dry and wet) of the samples was measured and subsequently, water uptake was calculated. Chondrocytes were seeded on either the scaffolds or polystyrene (PS), and cell proliferation was measured using MTT test.**Results:** PGA/PVA/CH showed significantly higher pH than other scaffolds, except of PVA/PVA/PVA. The PGA, and PGA/HA scaffolds showed significantly lower pH than other scaffolds. PVA/PVA demonstrated significantly higher, and PVA/PVA/PVA and PGA/HA II lower water uptake than other scaffolds. On the day 1 and 7, a significantly higher absorbance of PS, PGA/PVA, and PVA/PVA/PVA was found. However, the PVA/PVA/CH absorbance was at the same level as that of PS and PVA/PVA/PVA after 14 days. HA containing scaffolds showed a significantly lower MTT absorbance than other scaffolds.**Conclusions:** Non-woven scaffolds containing PGA, PVA, and chitosan demonstrated improved pH stability and cell proliferation compared to PGA scaffold. Therefore, they are promising for cartilage regeneration.**P118****Poly-ε-caprolactone/gel hybrid scaffolds for cartilage tissue engineering**J. Schagemann<sup>1</sup>, H. Chung<sup>2</sup>, J.J. Stone<sup>3</sup>, E. Mrosek<sup>4</sup>, J.S. Fitzsimmons<sup>3</sup>, T.J. Ruesink<sup>2</sup>, S.W. O'Driscoll<sup>2</sup>, G.G. Reinholz<sup>2</sup>;<sup>1</sup>Department Of Orthopedics, Mayo Clinic, Rochester, United States of America, <sup>2</sup>Cartilage & Connective Tissue Research, Mayo Clinic College of Medicine, Rochester, MN, United States of America, <sup>3</sup>Cartilage & Connective Tissue Research, Mayo Clinic College of Medicine, Rochester, MN, United States of America, <sup>4</sup>,**Purpose:** To determine the suitability of hybrid scaffolds composed of naturally derived biopolymer gels and macro-porous poly-ε-caprolactone (PCL) scaffolds for differentiated neo-cartilage formation in vitro.**Methods and Materials:** Rabbit articular chondrocytes were seeded into 1 wt% PCL/HA (hyaluronan), 0.5 wt% PCL/CS (chitosan), 1:3 PCL/F (fibrin sealant plus aprotinin), and 0.24% PCL/COL1 (type I collagen) hybrids, and were cultured statically for up to 50 days in vitro. Growth characteristics were evaluated by standard histology, scanning electron microscopy and confocal laser scanning microscopy. Neo-cartilage was quantified using a dimethyl-methylene blue assay for sulphated glycosaminoglycans (sGAG, n=14) and an enzyme linked immunosorbent assay for type II collagen (COL2, n=14), normalized to dsDNA content by the fluorescent picoGreen assay.**Results:** 1 hr after being seeded into scaffolds, predominantly spheroidal chondrocytes were homogeneously distributed throughout scaffolds' volume. Immunofluorescence depicted extending proteoglycan deposition with time. sGAG increased in all hybrids between day 25 and day 50. PCL/HA scaffolds consistently promoted highest yields. In contrast, total sGAG and total COL2 decreased in all hybrids except for PCL/CS, which favored increasing values and significantly higher total COL2 at day 50. dsDNA content decreased significantly over time and particularly within the first 12 days. Yet, PCL/HA displayed 2 proliferation peaks at days 3 and day 25.**Conclusions:** Developed hybrids create a propitious (short term) environment for implanted cells. PCL/HA and PCL/CS hybrids promote specific neo-cartilage formation and initial cell retention, and are thus particularly attractive for cartilage tissue engineering. Combined biopolymers can supply synthetic materials with inherent biomimetic and bioactive properties for biologic interactions with mammalian cells.**P119****Molecular biological properties of equine chondrocytes cultivated on 3D scaffold**W. Schlegel<sup>1</sup>, A. Berger<sup>2</sup>, C. Albrecht<sup>3</sup>, B. Lurf<sup>3</sup>, V. Zimmermann<sup>3</sup>, C. Meyer<sup>4</sup>, S. Marlovits<sup>5</sup>;<sup>1</sup>Department Of Traumatology, Medical University Vienna, Vienna, Austria, <sup>2</sup>Department For Traumatology, Medical University Vienna, Vienna, Austria, <sup>3</sup>Department Of Traumatology, Medical University of Vienna, Vienna, Austria, <sup>4</sup>Clinic And Polyclinic For Traumatology, University of Giessen, Giessen, Germany, <sup>5</sup>Vienna, Austria**Purpose:** The horse seems to be a promising animal model for cartilage repair. In this feasibility study we tested on 3D scaffolds whether equine chondrocytes from a monolayer culture have the ability to re-differentiate or not.**Methods and Materials:** After 20 days in monolayer culture equine chondrocytes from the talocrural joint (n=21) were transferred onto different 3D-scaffolds, consisting either of hyaluronan or collagen type I/III. Cells from collagen gel cultivation were not propagated in monolayer culture prior to the transfer into the gel. Real time PCR of Collagen type I and Collagen Type II was chosen to assess the molecular biological differentiation status.**Results:** Native equine chondrocyte samples show an average differentiation index (=Col II/Col I ratio) of  $2 \times 10^5$ . Cells, which were propagated in monolayer culture, diminish their differentiation index from  $2 \times 10^2$  on day 20 to  $6,0 \times 10^4$  on day 40. Results for the 3D-scaffolds, after 20 days, show an average differentiation index of  $3,0 \times 10^2$  for the hyaluronan matrices,  $1,4 \times 10^3$  for the collagen scaffolds and  $2,7 \times 10^2$  for the collagen gel.**Conclusions:** Scaffolds show different influence on the differentiation status of the cells. The differentiation index of cells on the collagen membrane is diminished, the index of cells on hyaluronan and collagen gel is similar to that of cells from a 20 days monolayer culture. In any case the index of chondrocytes, which were transferred to membranes, is higher than the index of chondrocytes, which were not removed from monolayer culture.